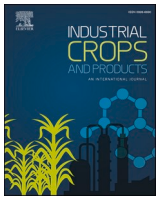
Industrial Crops & Products 228 (2025) 120868

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop



Antioxidant activity and neuromodulatory synergies in fixed oils from *Nigella sativa, Cucurbita pepo*, and *Sinapis alba Seeds*

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ARTICLE INFO

Keywords:
DPPH
AMPA
Nigella sativa
Cucurbita pepo
Sinapis alba
Combination
Cancer

ABSTRACT

Nigella sativa (black seeds), *Cucurbita pepo* (pumpkin), and *Sinapis alba* (mustard) are plants traditionally utilized for treating various ailments. This study aimed to extract fixed oils from the seeds of these plants and assess their antioxidant, antidiabetic, neuromodulatory, and anti-obesity properties. The effects of each oil were examined both individually and in combination through *in vitro* assays. The oil of *Nigella sativa, Cucurbita pepo,* and *Sinapis alba* seeds was extracted by cold pressing. Then, free radical scavenging, anti-α-amylase, and anti-lipase effects were investigated for all these fixed seeds. Electrophysiological recordings were done using the whole-cell patch clamp technique to assess the neuromodulatory activity. The percentage inhibition of DPPH (a free radical) was evaluated for fixed oils from *N. sativa*, *C. pepo*, and *S. alba*, as well as their combinations. *S. alba* oil demonstrated the highest antioxidant activity with an IC_{50} of 3.49 ± 0.22 μg/mL, followed by *C. pepo* oil at 7.97 ± 0.17 μg/mL. In contrast, *N. sativa* oil showed the lowest activity with an IC_{50} of 57.92 ± 0.32 μg/mL. The standard antioxidant, Trolox, had an IC_{50} of 2.02 ± 0.04 μg/mL. A combination of *C. pepo* and *S. alba* oils exhibited potent antioxidant properties, with an IC_{50} of 2.46 ± 0.03 μg/mL, comparable to Trolox. None of the oils significantly inhibited α-amylase or lipase enzymes. Electrophysiological assessments revealed that *S. alba* oil significantly reduced GluA2 and GluA2/3 receptor amplitude and influenced receptor kinetics ($p < 0.01$). *N. sativa* had a similar, though less pronounced, effect, while *C. pepo* showed no significant impact. Remarkably, a combination of *N. sativa* and *S. alba* oils synergistically decreased AMPA receptor amplitude and desensitization time, enhancing deactivation. This suggests a promising synergistic effect for neuromodulation beyond the individual impact of each oil. This study evaluated the antioxidant activity of *N. sativa, C. pepo,* and *S. alba* seeds fixed oils and their mixtures. *S. alba* oil showed the most potent antioxidant activity, followed by *C. pepo*. However, all tested oils showed weak inhibitory effects against lipase and α-amylase enzymes, suggesting weak potential for obesity and diabetic treatment. So, these natural fixed oils could be applied to prepare possible medications to prevent and treat oxidative stress.

1. Background

Oxidative stress is a major player in the pathogenesis of metabolic and neurodegenerative diseases. It is a byproduct of disrupting the equilibrium between the generation of free radicals and the body's ability to quench them with the support of antioxidants (Verma et al., 2020). The disruption of the equilibrium causes conditions such as obesity, diabetes, cardiovascular diseases, and neurological diseases like Parkinson's and Alzheimer's disease (Bet et al., 2006, Yudoh et al., 2005). Besides this, oxidative stress can also initiate chronic inflammation and tissue injury that accelerate the course of the disease. With its broad implications, the need to discover natural means to overcome oxidative injury is increasing (Akbarirad et al., 2016, Shebis et al., 2013).

In recent years, plant oils have also captured the attention of many due to their potential to confer several health benefits. The plant oils

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https://doi.org/10.1016/j.indcrop.2025.120868
Received 6 January 2025; Received in revised form 28 February 2025; Accepted 13 March 2025

have bioactive compounds with anti-inflammatory properties that are likely to present a solution to conditions associated with oxidative stress (Guo et al., 2020). Some types of plant oils have the potential to modulate metabolic functions, inhibit inflammation, and conserve neural pathways by evading oxidative stress (Li et al., 2020). All this has triggered research into their potential to avert or manage obesity, diabetes, and neurodegeneration (Akbarian et al., 2022).

Among the plant oils of various types, the cold-pressed oils of white mustard (Sinapis alba L.), pumpkin seed (Cucurbita pepo L.), and black seed (Nigella sativa) have received special interest. These are rich in bioactive compounds with several pharmacological activities, including antioxidative, anti-inflammatory, and neuroprotective activities (Martinović et al., 2020). White mustard is a rich phenolic acid sinapic acid source with an antioxidative activity of the free radicals of strong intensity. Pumpkin seed oil is rich in tocopherols, carotenoids, and polyunsaturated fatty acids that are the causes of antioxidative and anti-inflammatory activity (Nawirska-Olszańska et al., 2013, Rezig et al., 2012). Black seed oil is traditionally applied with medicines that is rich with thymoquinone with neuroprotective and metabolic regulator activity (Mukhtar et al., 2019, Kiralan et al., 2021).

One key pathway to neurodegeneration is the malfunction of the AMPA receptor (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor). AMPA receptors are involved in excitatory neurotransmission and synaptic plasticity that are necessary to learn and remember (Henley and Wilkinson, 2013). However, Under conditions of oxidative stress and excitotoxicity, AMPA receptor activity can malfunction with the influx of excessive amounts of calcium into the neuron with consequent neuronal injury (Henley and Wilkinson, 2013, Qneibi et al., 2024). Bioactive molecules of the bioactive compounds present in the oils can modulate AMPA receptor activity to produce protective actions. For example, sinapic acid present in white mustard can scavenge the free radicals to enhance the intracellular antioxidant defense to preserve the integrity of AMPA receptors (Wright and Vissel, 2012, Qneibi et al., 2024). the rich profile of the antioxidants present in pumpkin seed oil can inhibit glutamate-mediated excitotoxicity to avoid an excessive influx of cationic ions into the neuron (Ivica et al., 2024). Black seed oil thymoquinone can enhance the efficacy of the synapse and can provide protection against neuroinflammation to support the well-being of the neuron (Pereyra and Medina, 2021).

This research investigates the therapeutic benefits of cold-pressed oils from *N. sativa*, *C. pepo*, and *S. alba* on oxidative stress, diabetes, and obesity by assessing their antioxidant capacity and inhibitory effects on enzymes like lipase and amylase. *N. sativa*, *C. pepo*, and *S. alba* have been traditionally used for centuries in various cultures to treat various ailments, including metabolic disorders, neurological conditions, and oxidative stress-related diseases. Despite their widespread use in traditional medicine, there is limited scientific evidence exploring the combined effects of their fixed oils and their potential mechanisms of action. This study aims to address this gap by systematically evaluating these oils' antioxidant, antidiabetic, neuromodulatory, and anti-obesity properties, both individually and in combination, using in vitro assays. By investigating their synergistic effects, we sought to identify potential natural therapeutic agents that could complement or enhance existing treatments for oxidative stress, diabetes, obesity, and neurological disorders. Furthermore, this study provides a foundation for future research into developing plant-based therapies that are cost-effective, accessible, and aligned with traditional medicinal practices.

2. Material and methods

2.1. Materials and chemicals

All chemicals used in this study, including dimethyl sulfoxide (DMSO) (Riedel De Haen, Germany), Trolox ((s)-(-)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma Aldrich, Denmark), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, Germany), were of analytical grade. Pig pancreatic α-amylase was provided by MP Biochemicals (Illkirch, France), whereas acarbose, orlistat, starch, PNPP (p-nitrophenyl palmitate), and porcine pancreatic lipase were obtained from Sigma-Aldrich (USA). Only analytically graded materials were utilized in the tests. Dimethyl sulfoxide (DMSO) (Germany: Riedel De Haen).

2.2. Extraction of the fixed oils from Nigella sativa, Cucurbita pepo, and Sinapis alba seeds

Seeds of *N. sativa, C. pepo,* and *S. alba* were purchased from a herbalist in Jenin City in June 2023 and stored in dark, dry containers until extraction. Approximately 500 g of seeds were cold-pressed using a mini oil press machine (serial# 1510218A0002, Ltd., UK), following a previously established protocol (Wen et al., 2023). The yielded oils were kept separately in glass amber well-closed containers for further investigation.

2.3. Antioxidant activity for N. sativa, C. pepo, and S. alba seeds fixed oils

A methanolic stock solution of each fixed oil was prepared at 100 μg/mL and serially diluted to concentrations ranging from 5 to 100 μg/mL. For the assay, 1 mL of each dilution was mixed with 1 mL of methanol and 1 mL of 0.002 % DPPH solution. The mixtures were incubated at room temperature in the dark for 30 min, and absorbance was measured at 517 nm. The same procedure was repeated for Trolox as a positive control. A blank was prepared by mixing DPPH solution with methanol (1:1 ratio), and its absorbance was measured (Hawash et al., 2022a). The percentage of DPPH inhibition was calculated using the following equation:

$$\text{DPPH inhibition\%} = \frac{(\text{ABl}–\text{AS})}{\text{ABl}} X100\%$$

ABl: stated for blank absorbance.
AS: stated for sample absorbance.

2.4. α- Amylase enzyme inhibitory assay for antidiabetic activity evaluation

The α-amylase inhibitory assay was performed following a published protocol with minor modifications (Hawash et al., 2024). Each fixed oil was dissolved in 10 % DMSO and diluted with a buffer solution (Na2HPO4/NaH2PO4 (0.02 M) and NaCl (0.006 M), pH 6.9) to a concentration of 1000 μg/mL. Serial dilutions of 10, 50, 70, 100, and 500 μg/mL were prepared. A mixture of 0.2 mL porcine pancreatic α-amylase (2 units/mL) and 0.2 mL oil was incubated at 30 °C for 10 min. Then, 0.2 mL of 1 % starch solution was added, and the mixture was incubated for 3 min. The reaction was stopped by adding 0.2 mL dinitrosalicylic acid (DNSA), diluting with 5 mL distilled water, and heating at 90 °C for 10 min. After cooling, absorbance was measured at 540 nm. A blank was prepared by replacing the oil with 0.2 mL buffer. Acarbose was used as a positive control following the same procedure. The following equation was used to calculate the α-amylase inhibitory activity:

$$\text{Alpha amylase inhibitory\%} = \frac{(\text{ABl}–\text{AT})}{\text{ABl}} X100\%$$

ABl: absorbance of the blank solution.
AT: states for absorbance of oil sample solution.

2.5. Porcine Lipase inhibitory assay for anti-obesity evaluation

For each fixed oil and orlistat (positive control), fresh stock solutions were prepared at 1000 μg/mL and serially diluted to 50, 100, 200, 300, and 400 μg/mL. A 1 mg/mL porcine pancreatic lipase enzyme stock

solution was prepared in 10 % DMSO. For the assay, 0.2 mL of enzyme solution was mixed with 0.7 mL Tris-HCl buffer (pH 7.4, 0.1 M) and 0.1 mL of the test solution. A blank was prepared using 0.9 mL Tris-HCl and 0.1 mL enzyme solution without the inhibitor. All tubes were incubated at 37 °C for 15 min. After adding 0.1 mL of PNPB (100 mM in acetonitrile), the tubes were incubated for an additional 30 min. Pancreatic lipase activity was determined by measuring the hydrolysis of PNPB to p-nitrophenol at 410 nm using a UV–visible spectrophotometer (Jaradat et al., 2022).

The following equation was used to make calculations of the inhibition percentage:

$$\text{Inhibition}(\%) = \frac{(\text{Bl} - \text{S})}{\text{Bl}} X100\%$$

Where S states for the sample absorbance and Bl to the blank absorbance.

2.6. Cell viability analysis and anticancer effect test protocol

The extracted oils were tested in vitro for anticancer effects against six cancer cell lines: B16F1 (melanoma), HepG2 and Hep-3B (hepatocellular), HeLa (cervical), MCF-7 (breast), and CaCo-2 (colorectal). A normal cell line, HEK293T, was included for comparison. Cancer cell lines were cultured in RPMI-1640 medium, while the normal cell line was cultured in DMEM supplemented with 10 % fetal bovine serum, 1 % L-glutamine, and 1 % penicillin/streptomycin. All cells were maintained in a humidified atmosphere with 5 % CO_2 at 37 °C. Cells were seeded in 96-well plates at a density of 1000 cells per well. After 24 h, they were treated with various oil concentrations (1000, 500, 200, 100, and 50 μg/mL) for 72 h to determine IC_{50} values. Cell viability was assessed using the Cell Titer 96® Aqueous One Solution Cell Proliferation (MTS) Assay according to the manufacturer's instructions. Following treatment, 20 μL of MTS solution was added to each well containing 100 μL of media and incubated at 37 ∘C for 2 h. Absorbance was then measured at 490 nm to quantify cell viability (Hawash et al., 2022b).

2.7. Electrophysiology recordings

The AMPA receptor subunits in this research project were assembled in their flip isoform. Constructs of GluA2–3 (Q-form/flip) were provided by the Salk Institute, La Jolla, California (courtesy of S.F. Heinemann). HEK293T cells (Sigma, Germany) were used as the cellular model and cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma, USA) supplemented with 10 % fetal bovine serum, 0.1 mg/mL streptomycin, and 1 mM sodium pyruvate (Biological Industries in Beit-Haemek, Israel). Cells were incubated at 37 °C in a humidified atmosphere with 5 % CO_2.

The wild-type AMPAR DNA was inserted into HEK293T cells using the pRK5 plasmid, which was genetically modified to co-express enhanced green fluorescent protein, EGFP (Clontech, Palo Alto, California). Transfection was performed using jetPRIME (Polyplus, NY) with a 1:9 ratio of pEGFP-C1 to GluA components, following the manufacturer's instructions (Qneibi et al., 2019, Qneibi et al., 2020, Qneibi et al., 2022). After the incubation period of 36 h post-transfection, the cells were analyzed for their electrophysiological properties or observed under a stereomicroscope. Cells selected for recording were seeded onto Laminin-coated coverslips (1 mg/mL, Sigma, Germany) and identified by fluorescence intensity. Whole-cell patch-clamp recordings were performed using a patch-clamp amplifier and data acquisition system (Sutter Instruments, Novato, CA, USA). A dual-barrel theta glass pipette was actuated by a piezoelectric translator (Automate Scientific, Berkeley, CA, USA), facilitating rapid solution exchange during the experiment. Test solutions contained extracted essential oils from Sinapis alba (white mustard), Cucurbita pepo (pumpkin seeds), and Nigella sativa (black seeds), co-applied with 10 mM glutamate. The external recording solution consisted of 2.8 mM KCl, 150 mM NaCl, 2 mM CaCl2, 0.5 mM MgCl2, and 10 mM HEPES, pH 7.4 with NaOH. Patch electrodes were made from borosilicate glass and filled with an internal solution containing 110 mM CsF, 30 mM CsCl, 4 mM NaCl, 0.5 mM $CaCl_2$, 10 mM EGTA, and 10 mM HEPES (pH 7.2, CsOH-adjusted). Electrode resistance ranged between 2–4 MΩ. Junction potentials were measured post-recording, with a solution exchange rise time of 200–300 microseconds (10–90 %). Accordingly, deactivation and desensitization currents were elicited by applying 10 mM glutamate for 1 ms and 500 ms at a holding potential of −60 mV in solutions in a pH 7.4 and room temperature conditions (20–23 °C). These currents were recorded at a high sampling frequency of 10 kHz, low-pass filtered at 2 kHz to reduce high-frequency noise, and digitized with SutterPatch Software v. 1.1.1 from Sutter Instruments. A total of 10 patch cells were used for statistical reliability. Oil concentrations were determined by incremental testing, starting at 100 μM and increasing until the maximum effect was observed without compromising cell viability, which was 600 μg/mL. In combination studies, essential oils were mixed in a 1:1 ratio (50 % of each oil).

2.8. Statistical analysis

All data were reported as mean values with standard deviations (SD), and statistical significance was established with a p-value threshold of less than 0.05. The experimental works were conducted in triplicates. The analysis was performed using an unpaired *t*-test to evaluate the differences between groups and determine the significance of the findings.

3. Results

3.1. Free radicals scavenging activity

Table 1 and Fig. 1 present the percentage inhibition of DPPH by *Nigella sativa* (black seeds), *Cucurbita pepo* (pumpkin seeds), and *Sinapis alba* (mustard seeds) fixed oils, along with their mixtures at various concentrations. Among the tested oils, *S. alba* demonstrated the most potent antioxidant activity, with an IC_{50} of 3.49 ± 0.22 μg/mL, closely followed by *C. pepo* (IC_{50} = 7.97 ± 0.17 μg/mL). In contrast, *N. sativa* exhibited the weakest antioxidant activity (IC_{50} = 57.92 ± 0.32 μg/mL). The combination of *C. pepo* and *S. alba* oils showed remarkable antioxidant potential, with an IC_{50} of 2.46 ± 0.03 μg/mL, nearly matching the reference compound Trolox (IC_{50} = 2.02 ± 0.04 μg/mL). The *N. sativa* and *S. alba* mixture displayed moderate activity (IC_{50} = 9.63 ± 0.15 μg/mL), while the *N. sativa* and *C. pepo* blend had the weakest antioxidant effect among the mixtures (IC_{50} = 13.82 ± 0.63 μg/mL).

The strong antioxidant activity of *S. alba* oil aligns with previous studies, which attribute its potency to bioactive compounds such as 3,4-dihydroxybenzoic acid, ferulic acid, sinapic acid, and rutin (Efrem et al., 2022). These compounds are known for their free radical scavenging properties, making *S. alba* oil a promising natural antioxidant. Similarly,

Table 1
Compared with the positive controls, the IC_{50} values for all evaluated oils and mixture oils against DPPH, amylase, and lipase enzymes.

	IC_{50} μg/mL		
	DPPH	Amylase	Lipase
Black seeds	57.92 ± 0.32	> 500	> 500
Pumpkin oil	7.97 ± 0.17	> 500	> 500
Mustard oil	3.49 ± 0.22	> 500	> 500
Black+Pumpkin	13.82 ± 0.63	> 500	> 500
Black seeds +Mustard	9.63 ± 0.15	> 500	> 500
Pumpkin+Mustard	2.46 ± 0.03	> 500	> 500
Positive control	2.02 ± 0.04[a]	6.04 ± 0.45[b]	21.82 ± 2.07[c]

[a] Trolox,
[b] Acarbose,
[c] Orlistat

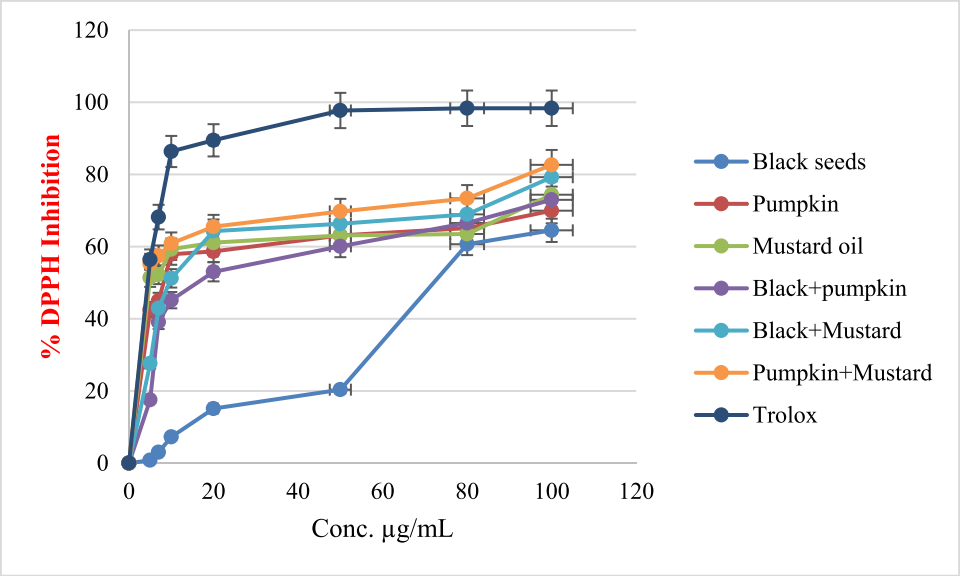

Fig. 1. DPPH free radical scavenging activity by *Nigella sativa* (black seeds), *Cucurbita pepo* (pumpkin seeds), *Sinapis alba* (mustard seeds), and their combinations, besides the positive control.

the antioxidant activity of *C. pepo* oil can be linked to its high content of unsaturated fatty acids, including linoleic acid (42.1–48.5 %) and oleic acid (18.4–39.6 %), which are well-documented for their antioxidant effects (Benalia et al., 2015). In contrast, the relatively weak antioxidant activity of *N. sativa* oil may be due to its lower concentration of bioactive compounds compared to the other oils. However, *N. sativa* is still recognized for its thymoquinone content, which, while not dominant in this study, has been shown to exhibit antioxidant properties in other contexts. These findings highlight the potential of *S. alba* and *C. pepo* oils as natural antioxidants and emphasize the importance of their bioactive constituents in combating oxidative stress.

3.2. Anti-α-amylase activity

Table 1 and Figs. 2 and 3 demonstrate the percentage of inhibition for α-amylase by *N. sativa* (black seeds), *C. pepo* (pumpkin seeds), and *S. alba* (mustard seeds) fixed oils, along with their mixtures at different concentrations. The results revealed that all tested oils exhibited a weak inhibitory effect on α-amylase, with IC_{50} values exceeding 500 µg/mL. This suggests these oils possess minimal potential for inhibiting α-amylase, making them unlikely candidates for effective diabetic treatment.

oils and their mixtures in comparison with Acarbose positive control at 100 µg/mL concentrations

Similarly, various mixtures of the oils were tested, and none demonstrated a significant inhibitory effect against α-amylase, with all mixtures yielding IC_{50} values of more than 500 µg/mL. These results align with prior studies that reported a lack of anti-amylase activity in *N. sativa*, *C. pepo*, and *S. alba* oils.

3.3. Anti-lipase activity

Table 1 and Figs. 4 and 5 present the inhibition percentage of lipase by *N. sativa* (black seeds), *C. pepo*, and *S. alba* fixed oils and their

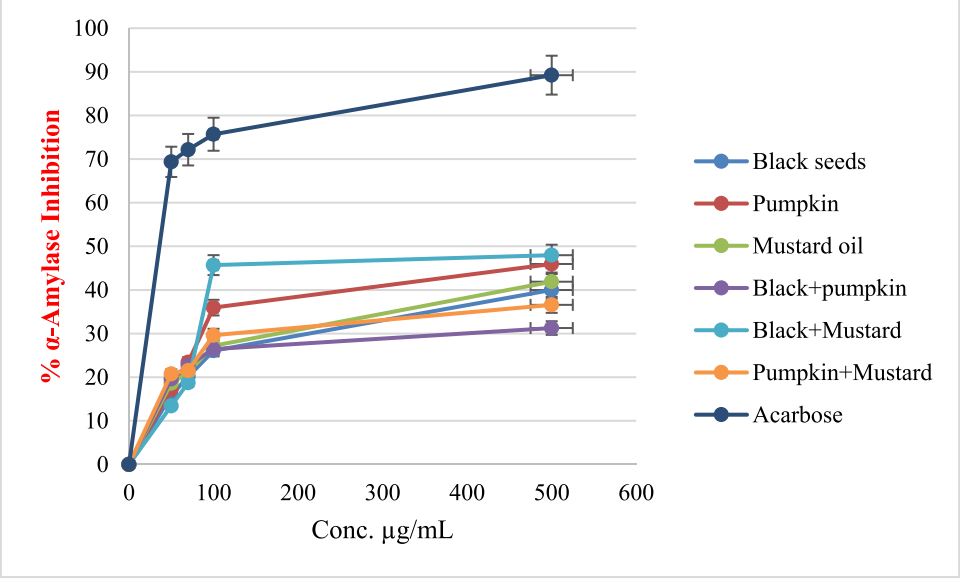

Fig. 2. Amylase inhibitory effects of all oils and their mixtures in comparison with Acarbose positive control.

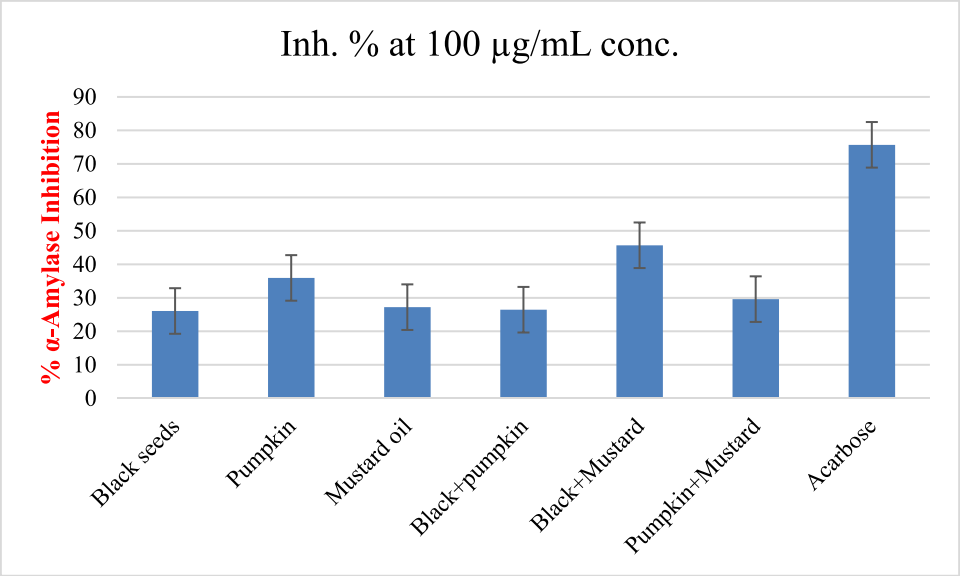

Fig. 3. Amylase inhibitory effects of all.

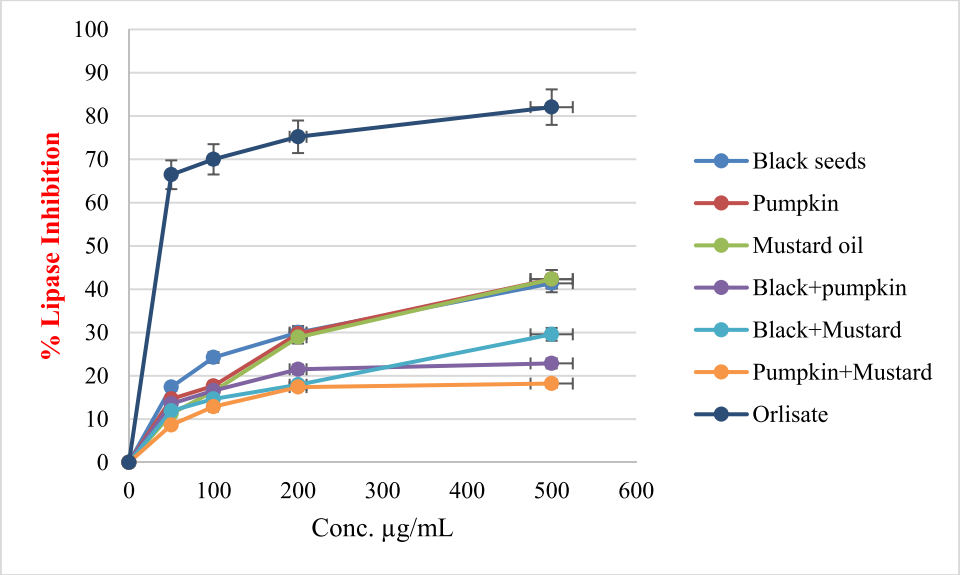

Fig. 4. Lipase inhibitory effects of all oils and their mixtures compared with orlistat positive control.

combinations, tested at different concentrations. All tested fixed oils exhibited weak inhibitory effects against lipase, with IC_{50} values exceeding 500 µg/mL, indicating limited potential in combating obesity through lipase inhibition.

A combination of these oils was also assessed, but none demonstrated significant lipase inhibition, as all combinations produced IC_{50} values of more than 500 µg/mL. These results are consistent with previous findings, which reported no notable anti-lipase activity for these oils.

The weak anti-α-amylase and anti-lipase activities of *N. sativa, C. pepo,* and *S. alba* oils, as evidenced by IC_{50} values exceeding 500 µg/mL, align with previous research (Gholamhoseinian et al., 2010) (Gupta and Serva Peddha, 2024). These findings suggest that the oils' bioactive compositions may lack sufficient concentrations or types of compounds required for effective enzyme inhibition. For instance, while these oils are rich in antioxidants and unsaturated fatty acids, they may not contain significant amounts of polyphenols or flavonoids, which are often associated with strong enzyme inhibitory effects. Additionally, while preserving heat-sensitive compounds, the cold-pressing extraction method may not extract certain bioactive molecules responsible for enzyme inhibition.

The lack of significant effects in oil mixtures further supports the idea that synergistic interactions, observed in antioxidant and neuromodulatory activities, do not extend to enzyme inhibition. This could be due to the absence of complementary compounds or insufficient concentrations of bioactive molecules in the mixtures. Future studies could explore the fractionation and isolation of specific compounds, alternative extraction methods, and in vivo experiments to better understand the potential of these oils in managing diabetes and obesity.

3.4. *Anticancer activity*

The cell viability percentages at 1 mg/mL for *N. sativa* (black seeds), *C. pepo* (pumpkin seeds), and *S. alba* (mustard seeds) fixed oils, along with their combinations, were evaluated across six cancer cell lines:

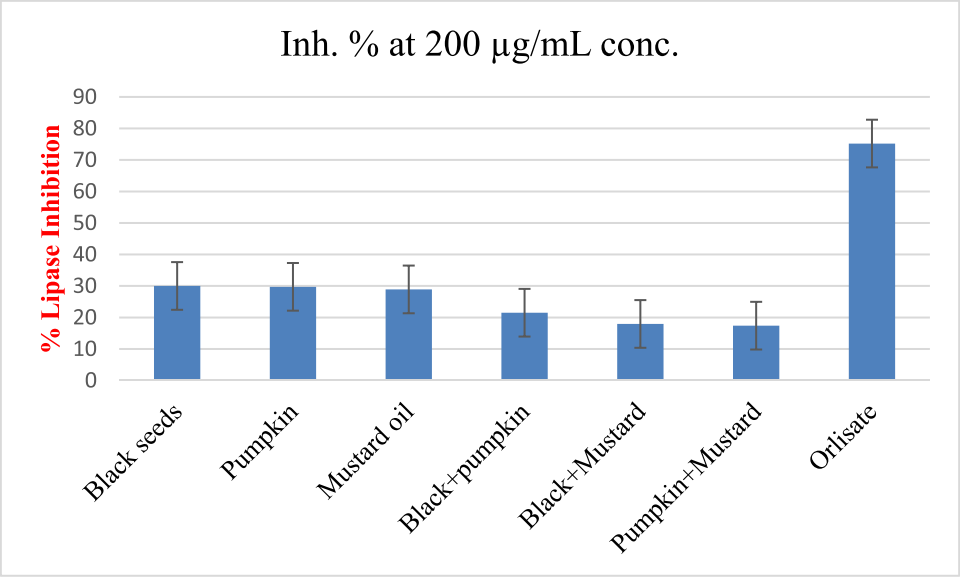

Fig. 5. Lipase inhibitory effects of all oils and their mixtures in comparison with Orlistat positive control at 200 µg/mL concentrations.

CaCo-2, HeLa, MCF-7, HepG2, B16F1, and Hep3B. Fig. 6 shows that Doxorubicin (DOX), used as the positive control, demonstrated nearly complete inhibition of cell viability in all the cancer cell lines, as expected for a potent chemotherapy drug. *N. sativa* oil exhibited moderate anticancer activity among the tested oils, resulting in approximately 40–50 % inhibition across all cell lines. *C. pepo* oil demonstrated slightly higher inhibition rates, ranging from 50–70 %, indicating stronger anticancer potential than *N. sativa* oil.

S. alba oil showed the most potent anticancer effects among the three oils, with inhibition rates exceeding 70 % in most of the tested cell lines, particularly in CaCo-2 and HepG2 cells. Additionally, combinations of *N. sativa* with *C. pepo* and *N. sativa* with *S. alba* (black + mustard) demonstrated enhanced anticancer effects, with inhibition percentages exceeding 80 % in HeLa and HepG2 cell lines. The mixture of *C. pepo* and *S. alba* (pumpkin + mustard) also showed strong anticancer activity, with inhibition rates comparable to those of the individual mustard oil, suggesting potential synergistic effects. Dimethyl sulfoxide (DMSO), the solvent control, exhibited no significant cytotoxicity, confirming that the observed inhibition was specifically due to the tested oils.

The potent anticancer activity of *S. alba* oil, particularly in CaCo-2 and HepG2 cell lines, may be attributed to its unique bioactive compounds, such as glucosinolates (e.g., sinigrin) and their hydrolysis products (e.g., allyl isothiocyanate) (Torrijos et al., 2023, Boscaro et al., 2018). These compounds are known to exert anticancer effects through multiple mechanisms. For instance, allyl isothiocyanate has been shown to induce apoptosis by activating both intrinsic and extrinsic apoptotic pathways, upregulating pro-apoptotic proteins like Bax, and downregulating anti-apoptotic proteins like Bcl-2. Additionally, *S. alba* compounds may induce cell cycle arrest, particularly at the G2/M phase, preventing cancer cell proliferation. Furthermore, these compounds can inhibit angiogenesis and metastasis by suppressing key signaling pathways such as VEGF and MMPs, which are critical for tumor growth and spread. Lastly, the generation of reactive oxygen species (ROS) by *S. alba* compounds can lead to oxidative damage in cancer cells, triggering cell

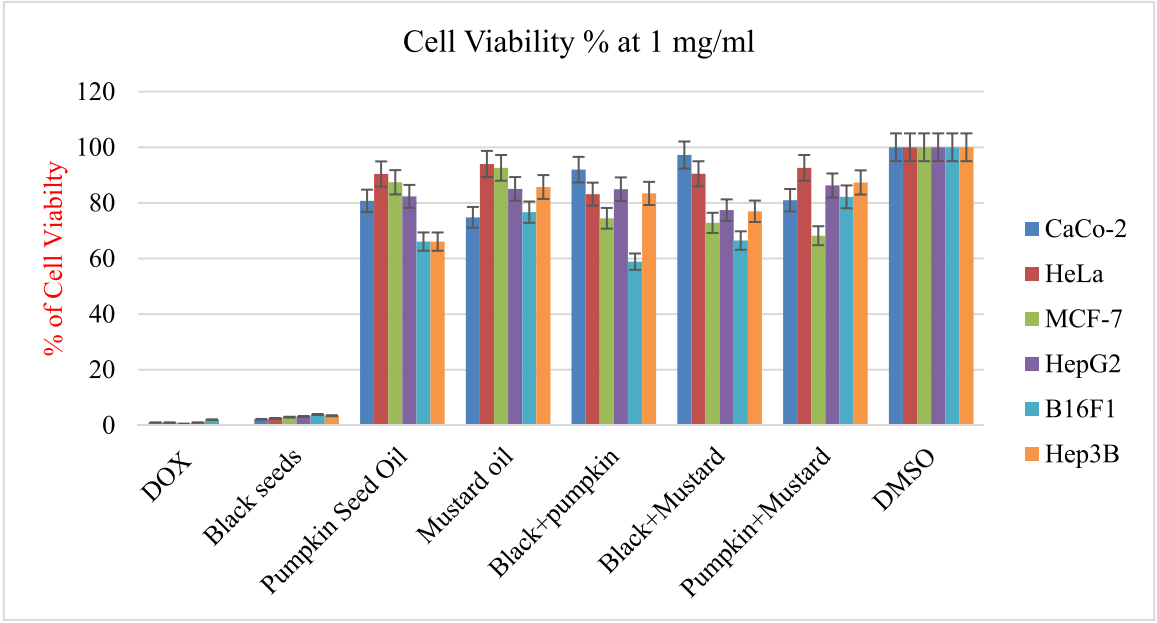

Fig. 6. Anticancer activity of *Nigella sativa*, *Cucurbita pepo*, and *Sinapis alba* fixed oils against six cancer cell lines.

death while sparing normal cells due to their higher antioxidant capacity.

3.5. Whole-cell patch clamp recordings

Whole-cell patch-clamp recordings were performed to evaluate the effects of *N. sativa*, *C. pepo*, and *S. alba* on the AMPA receptor subtypes GluA2 and GluA2/3. As shown in Fig. 7, For *N. sativa* (Table S1), the amplitude of the GluA2 receptor decreased by 3.15-fold when compared to the glutamate-alone condition (**$p < 0.01$). The amplitude returned to baseline levels after *N. sativa's* application and subsequent glutamate reapplication. Additionally, the deactivation time (t deact) increased 2.04-fold (**$p < 0.01$), while the desensitization time (t des) decreased 1.79-fold (**$p < 0.01$). A similar pattern was observed for the GluA2/3 receptor, with amplitude showing a 3.06-fold reduction (**$p < 0.01$), accompanied by a 1.8-fold increase in t deact and a 1.69-fold reduction in t des (**$p < 0.01$) (Fig. 8).

Fig. 7 shows the inhibitory effects of *N. sativa* (a, b), *C. pepo* (c, d), and *S. alba* (e, f) on the peak currents of GluA2 and GluA2/3 AMPA receptors. Whole-cell patch-clamp recordings were conducted to measure the amplitude of currents in the presence of glutamate (Glu) and after co-application with the respective oils. In panels a, c, and e, black bars represent the control (Glu-alone), and white bars represent Glu + oil treatments. *N. sativa* and *S. alba* significantly reduced the peak current amplitudes of both GluA2 and GluA2/3 receptors, whereas *Cucurbita pepo* had no significant inhibitory effect (ns: not significant). Panels b, d, and f show the amplitude ratios (A/A_1), further highlighting the significant inhibition by *N. sativa* and *S. alba* (**, *** $p < 0.01$, $p < 0.001$, respectively). Data are presented as mean ± SD ($n = 10$).

In the case of *Cucurbita pepo* (Table S2), As shown in Fig. 7, the GluA2 receptor amplitude decreased by 1.08-fold, though this reduction was not statistically significant (ns). Both the deactivation and desensitization times remained unchanged (Fig. 8). For GluA2/3 receptors, a 1.01-fold reduction in amplitude was observed (ns), with no significant changes in t deact or t des, indicating that *C. pepo* did not significantly affect the receptor kinetics (Fig. 8).

Fig. 8 shows the effects of *N. sativa*, *C. pepo*, *S. alba*, and their combinations on the desensitization (τ_w des, panels a–d) and deactivation (τ_w deact, panels e–h) times for GluA2 and GluA2/3 AMPA receptors. In panels (a) and (b), *Nigella sativa* and *Sinapis alba* significantly reduced desensitization times (**$p < 0.01$), while *C. pepo* showed no significant effect (ns). The combinations of *N. sativa* with *C. pepo* and *S. alba* further reduced desensitization times, as seen in panels (c) and (d), with *N. sativa* + *S. alba* showing the most potent reduction (***$p < 0.001$). In panels (e) and (f), deactivation times were significantly increased by *N. sativa* and *S. alba* (**$p < 0.01$), while *C. pepo* had no significant effect. Combinations of the oils, as shown in panels (g) and (h), resulted in more pronounced increases in deactivation times, with *N. sativa* + *S. alba*

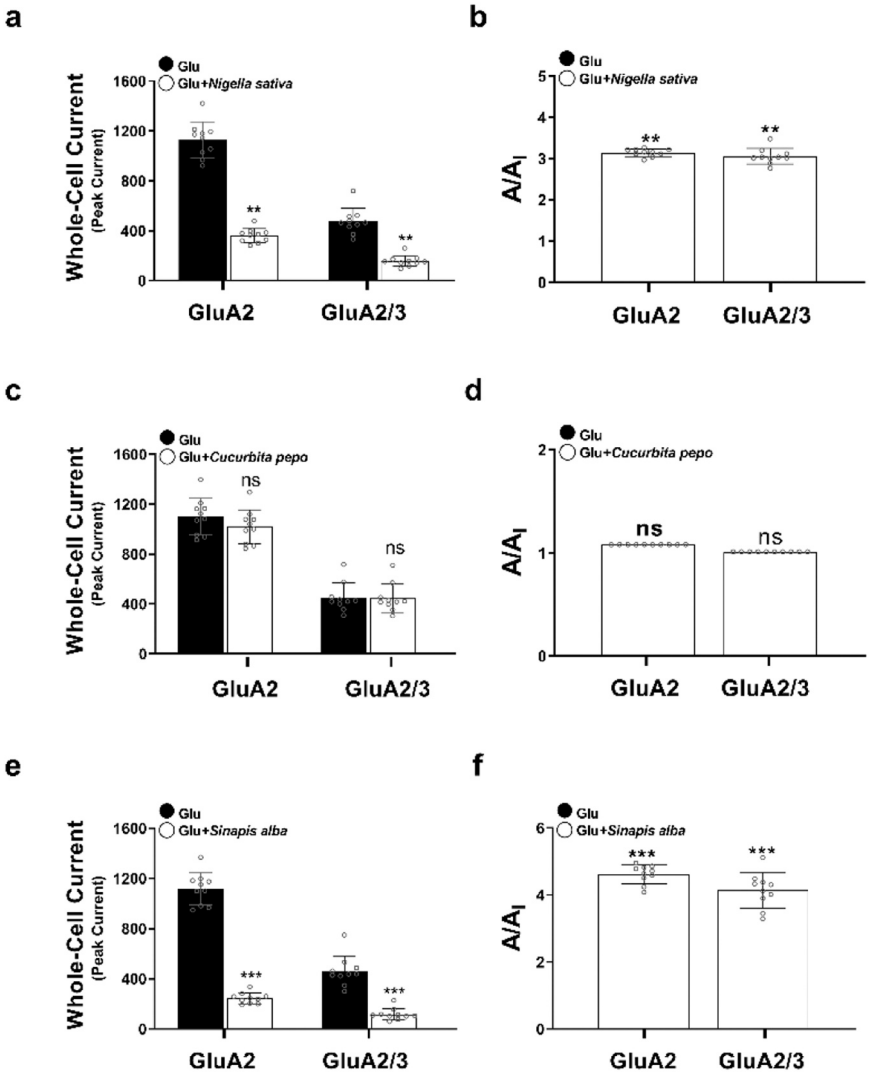

Fig. 7. Inhibitory Effects of *Nigella sativa*, *Cucurbita pepo*, and *Sinapis alba* on AMPA Receptor Currents. *P* value > 0.05 is considered significant.

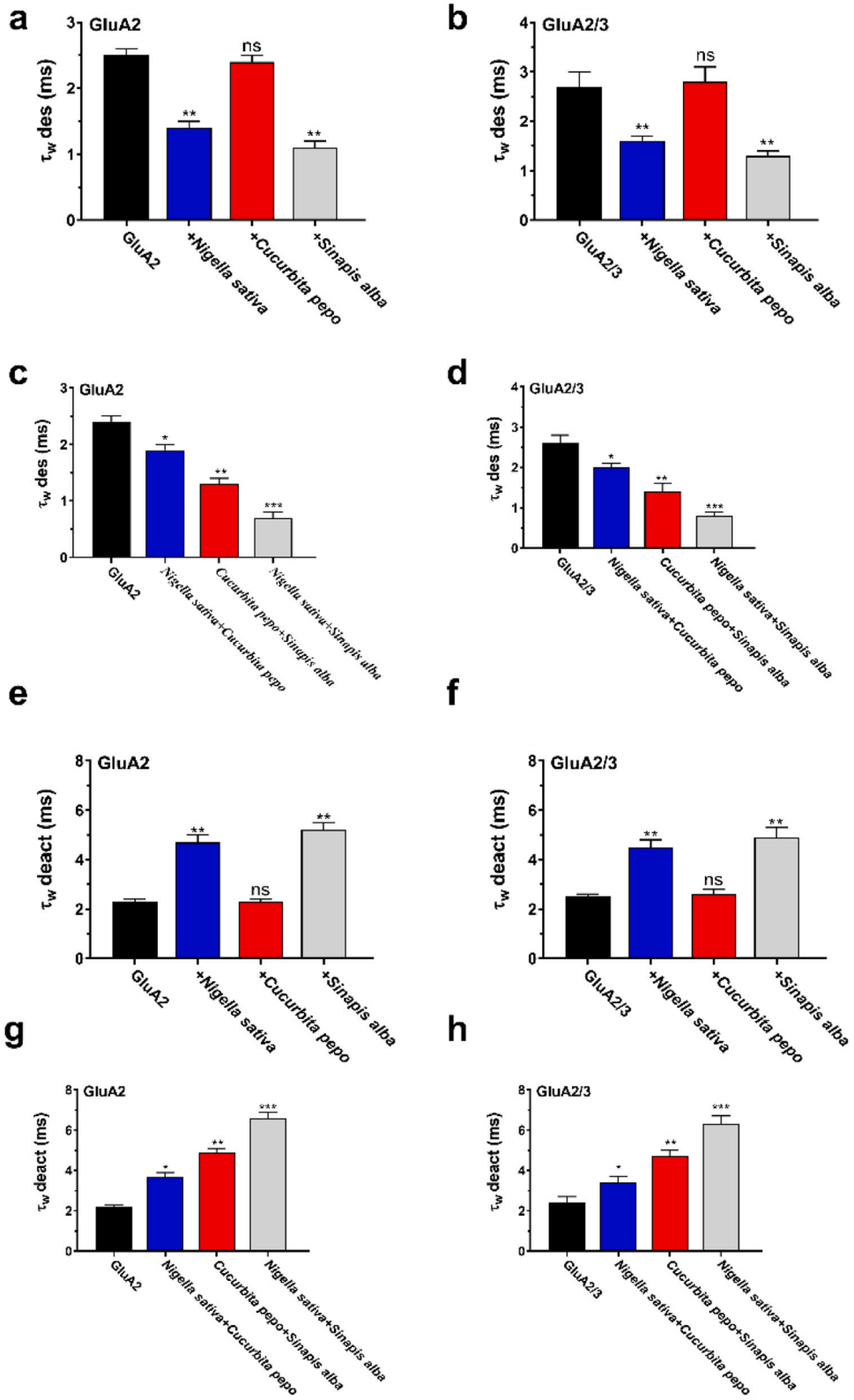

Fig. 8. Effects of *Nigella sativa*, *Cucurbita pepo*, and *Sinapis alba* and Their Combinations on Desensitization and Deactivation Times of AMPA Receptors. *P* value > 0.05 is considered significant.

showing the largest effect (***$p < 0.001$). Data are presented as mean $\pm$ SD ($n = 10$), with *$p < 0.05$, * *$p < 0.01$, * **$p < 0.001$ indicating statistical significance.

S. alba (Table S3) demonstrated the most significant effect on receptor activity. As shown in Fig. 7, The amplitude of the GluA2 receptor decreased by 4.62-fold (***$p < 0.001$), while the deactivation time increased by 2.26-fold (**$p < 0.01$), and the desensitization time decreased by 2.27-fold (**$p < 0.01$) (Fig. 8). Similarly, GluA2/3 receptor amplitude decreased by 4.14-fold (***$p < 0.001$), with a 1.96-fold increase in t deact and a 2.15-fold reduction in t des (**$p < 0.01$).

These results suggest that *S. alba* significantly alters the amplitude and kinetics of AMPA receptor activity (Fig. 8).

Further combinations of the oils also exhibited significant effects on AMPA receptor function. The co-application of *N. sativa* and *C. pepo* (Table S4) reduced the GluA2 receptor amplitude by 2.33-fold (*p < 0.05) (Fig. 9), with an accompanying 1.68-fold increase in t deact (*p < 0.05) and a 1.26-fold reduction in t des (*p < 0.05). A similar effect was observed for GluA2/3 receptors, with a 2.17-fold decrease in amplitude (**p < 0.01), along with a 1.42-fold increase in t_deact (*p < 0.05) and a 1.3-fold reduction in t_des (*p < 0.05). These results suggest combining *N. sativa* with *C. pepo* amplifies the modulatory effects on receptor function (Fig. 8).

Fig. 9 illustrates the effects of combining *N. sativa*, *C. pepo*, and *S. alba* on the peak whole-cell currents and current ratios (A/A_1) for GluA2 and GluA2/3 AMPA receptors. In panels (a) and (b), the combination of *N. sativa* and *C. pepo* significantly reduced the peak currents of both receptor subtypes (*p < 0.05), with corresponding decreases in the A/A_1 ratios. Panels (c) and (d) show the effects of the combination of *C. pepo* and *S. alba*, which produced even more pronounced inhibition of GluA2 and GluA2/3 currents (**p < 0.01). The most significant inhibition was observed with the combination of *N. sativa* and *S. alba* (panels e and f), resulting in a substantial decrease in peak currents and current ratios (***p < 0.001) for both receptor subtypes. Data are presented as mean ± SD (n = 10), with *p < 0.05, * *p < 0.01, * **p < 0.001 indicating statistical significance.

The combination of *C. pepo* and *S. alba* (Table S5) resulted in a more pronounced inhibition, with the amplitude of the GluA2 receptor decreasing by 3.25-fold (**p < 0.01) (Fig. 9). At the same time τ deact increased by 2.23-fold (**p < 0.01). Similarly, the amplitude of GluA2/3 receptors decreased by 2.96-fold (**p < 0.01), with a significant increase in deactivation time (*p < 0.01), indicating a strong inhibitory effect on receptor kinetics (Fig. 8).

The most pronounced effects were observed with the combination of *N. sativa* and *S. alba* (Table S6), where the amplitude of the GluA2 receptor was reduced by 5.75-fold (***p < 0.001) (Fig. 9), with a 2.98-fold increase in τ deact (***p < 0.001) and a 3.43-fold reduction in τ des (***p < 0.001). Similar effects were observed for GluA2/3 receptors, where the amplitude was reduced by 5.41-fold (***p < 0.001), with significant alterations in deactivation and desensitization times (***p < 0.001), indicating that this combination exerts the most substantial modulation on AMPA receptor kinetics and function (Fig. 8).

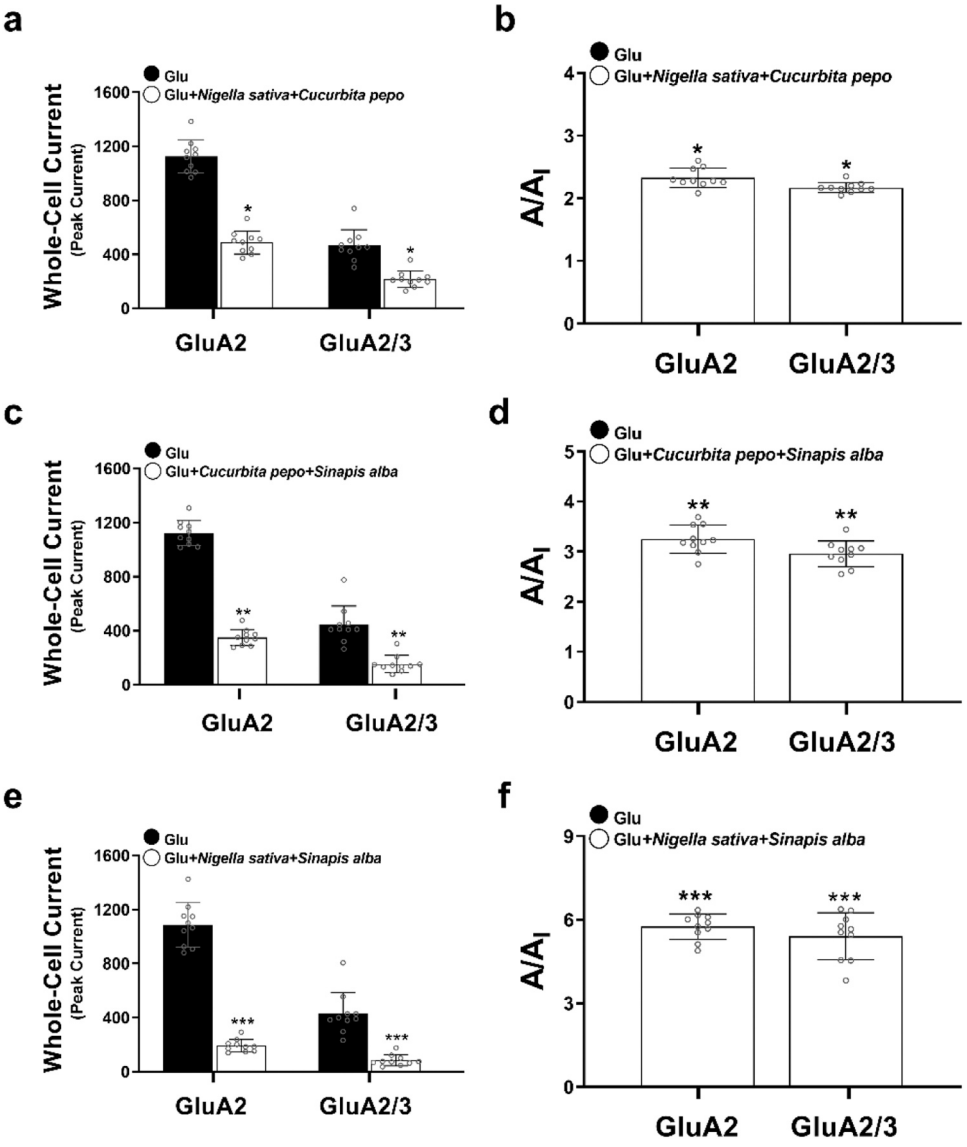

Fig. 9. Combined Effects of *Nigella sativa*, *Cucurbita pepo*, and *Sinapis alba* on AMPA Receptor Whole-Cell Currents. *P* value > 0.05 is considered significant.

4. Discussion

The findings of this study demonstrate the diverse bioactivities of *N. sativa*, *C. pepo*, and *S. alba* seed oils, focusing on their antioxidant, enzyme inhibition, anticancer, and neuromodulatory properties. Collectively, these results highlight the therapeutic potential of these natural oils, although their effectiveness varies based on the specific biological activity examined (Jaradat et al., 2023). Each oil presents a unique bioactivity profile, indicating its potential role in various medical applications.

The free radical scavenging activity assay results demonstrate that *S. alba* oil is the most potent antioxidant of the three oils tested, consistent with earlier studies. As reported by G. D. Haki, mustard seeds contain several strong antioxidant compounds, such as 3,4-dihydroxybenzoic acid, ferulic acid, sinapic acid, and rutin (Efrem et al., 2022). These compounds likely contribute to the powerful antioxidant effect observed in *S. alba* oil, reflected by its IC_{50} value being nearly equal to that of the reference compound Trolox. The presence of these compounds underscores mustard oil's potential as a natural antioxidant, providing a robust defense against oxidative stress. The second most effective oil, *C.pepo*, aligns with previous research conducted by Mohamed Yousfi, which showed that pumpkin seeds are rich in unsaturated fatty acids, including linoleic acid (42.1–48.5 %) and oleic acid (18.4–39.6 %). These fatty acids are well-known for their potent antioxidant properties (Benalia et al., 2015). The presence of these compounds likely explains the strong antioxidant activity exhibited by pumpkin seed oil in this study. The relatively high IC_{50} value of *Nigella sativa* oil suggests that it is less effective as an antioxidant while still containing bioactive components than mustard and pumpkin oils.

These findings are further supported by comparative data from different regions, summarized in Table 2, which shows some variation in the IC_{50} values for the oils. For example, *N. sativa* oils from Turkey, Iran, Jordan, and Sudan exhibit antioxidant activities that vary in potency but do not match the effectiveness of mustard and pumpkin oils. *C. pepo* oils from China, Turkey, Malaysia, and Sudan similarly show variations in their antioxidant capacities, suggesting that regional differences and extraction methods may impact the efficacy of these oils as antioxidants.

While the oils demonstrated potent antioxidant properties, their ability to inhibit key metabolic enzymes like amylase and lipase was limited. As shown in Table 3, all tested oils and mixtures exhibited weak inhibitory effects against amylase, with IC_{50} values exceeding 500 μg/mL. This suggests that these oils have minimal potential as antidiabetic agents, where inhibiting amylase is critical to controlling the breakdown of carbohydrates and thus regulating blood sugar levels.

For comparison, the anti-amylase activity of these oils from different regions, as summarized in Table 3, shows that *N. sativa* oils from Turkey exhibit around 50–60 % amylase inhibition. In contrast, *C. pepo* oil from China and India showed IC_{50} values of 40.68 μg/mL and 138.22 μg/mL, respectively. However, in this study, none of the oils reached such levels of inhibition, underscoring the variation in oil bioactivity across regions. *S. alba* oil from Egypt demonstrated some inhibition with an IC_{50} of 360 μg/mL but still falls short of the necessary potency for therapeutic applications.

Similarly, the oils demonstrated weak inhibition against lipase, as shown in Table 4, with IC_{50} values exceeding 500 μg/mL for all oils and mixtures tested. This indicates that the oils have limited potential in obesity treatment, where lipase inhibition is crucial to prevent the breakdown and absorption of fats. Mustard oil, which demonstrated some lipase inhibition in studies from Palestine with an IC_{50} of 296.87 μg/mL, suggests some regional variability in bioactivity. Nonetheless, the overall results suggest these oils are not strong candidates for lipase inhibition.

The anticancer results provide another dimension to the therapeutic potential of these oils. Among the tested oils, *Sinapis alba* once again stood out for its potent anticancer effects, showing high inhibition rates across several cancer cell lines, particularly in CaCo-2 and HepG2 cells. This suggests a possible correlation between its strong antioxidant properties and its ability to induce cytotoxicity in cancer cells, as oxidative stress is a key player in cancer cell proliferation and survival.

The tested oils, particularly *S. alba* and its combinations show promise as complementary agents to conventional cancer therapies. For example, the pro-apoptotic and oxidative stress-inducing effects of *S. alba* oil could enhance the efficacy of chemotherapy drugs like doxorubicin by sensitizing cancer cells to treatment. This is particularly relevant for cancers that develop resistance to chemotherapy, as natural compounds like those in *S. alba* oil may help overcome drug resistance mechanisms. Additionally, the antioxidant properties of these oils could mitigate the side effects of chemotherapy, such as oxidative damage to healthy tissues, while selectively targeting cancer cells. For instance, the combination of *N. sativa* and *S. alba* oils could be explored as an adjunct therapy to reduce chemotherapy-induced toxicity and improve patient outcomes.

The combination of oils, particularly *black + mustard* and *pumpkin + mustard* mixtures, also showed enhanced anticancer effects, indicating possible synergistic interactions between the bioactive compounds present in these oils. These findings are promising, as they suggest that combining different natural oils could enhance their anticancer potential. However, the anticancer effects of these oils were moderate compared to the potent chemotherapy drug doxorubicin (DOX), which served as the positive control in this study. Therefore, while these oils may have potential as complementary treatments in cancer therapy, they are unlikely to replace conventional anticancer drugs.

Table 2
Antioxidant IC_{50} for *N. Sativa*, *C. pepo*, and *S. alba* seeds fixed oils from different world regions.

DPPH assay	% of inhibition or IC_{50}	Region	Ref.
***N. Sativa* seeds**	52.61 ± 0.22 μg/mL	Turkey	(Sicak and Eliuz, 2019)
	12.6 ± 0.0 μg/mL	Iran	(Kazemi, 2015)
	32.32 μg/mL	Jordan	(Sultan et al., 2009)
	4.48 mg.mL	Sudan	(Haron et al., 2014)
***C. pepo* oil**	123.93 mg/mL	China	(Jiao et al., 2014)
	46.53 % DPPH inhibition	Turkey Malaysia	(şengün et al., 2021) (Kadir et al., 2024)
	142.857 μg/mL. 0.1 mg/mL	Sudan	(Abadi and Ahmed)
***S. alba* oil**	57.13 % DPPH inhibition	Canada Pakistan	(Imran, 2021) (Anwar et al., 2013)
	25 μg/mL	India	(Chaudhary et al., 2016)
	2750 μg/mL		

Table 3
Anti-amylase IC_{50} for *N. sativa*, *C. pepo*, and *S. alba* seeds fixed oils from different world regions.

Anti-α-amylase assay	% of inhibition or IC_{50}	Region	Ref.
***N. sativa* seeds**	50–60 % amylase inhibition	Turkey	(Gupta and Serva Peddha, 2024)
***C. pepo* oil**	40.68 μg/mL	China	(Li et al., 2016)
	138, 22 μg/mL	India	(Monica et al., 2022)
***S. alba* oil**	360 μg/mL	Egypt	(Salah et al., 2024)

Table 4
Anti-lipase IC_{50} for *N. sativa*, *C. pepo*, and *S. alba* seeds fixed oils from different world regions.

Anti-lipase assay	% of inhibition or IC_{50}	Region	Ref.
***N. sativa* oil**	50–60 % lipase inhibition	Turkey Review	(Gupta and Serva Peddha, 2024)
	20–50 % lipase inhibition		(Gholamhoseinian et al., 2010)
***C. pepo* oil**	No data	-	-
***S. alba* oil**	296.87 μg/mL	Palestine	(Jaradat et al., 2017)

The selective anticancer effects of *S. alba* oil, particularly its strong inhibition in CaCo-2 (colorectal cancer) and HepG2 (hepatocellular carcinoma) cells, may be due to differences in these cancer types' molecular and metabolic profiles. For example, CaCo-2 and HepG2 cells often exhibit high levels of oxidative stress and altered apoptotic signaling, making them more susceptible to the pro-oxidant and pro-apoptotic effects of *S. alba* compounds. In contrast, other cell lines, such as HeLa (cervical cancer) and MCF-7 (breast cancer), may have different resistance mechanisms or lower sensitivity to the specific bioactive compounds in S. alba oil. This selectivity highlights the potential of *S. alba* oil as a targeted therapeutic agent for specific cancer types, particularly those with high oxidative stress and dysregulated apoptosis.

The effects of *N. sativa*, *C. pepo*, and *S. alba* on AMPA receptors further expand our understanding of their modulatory potential. The whole-cell patch-clamp recordings revealed that *Nigella sativa* and *Sinapis alba* significantly reduced the amplitude of GluA2 and GluA2/3 receptor responses. *S. alba* showed the most pronounced effects, decreasing receptor amplitude over 4 fold. These results suggest a possible modulation of AMPA receptor function, which is crucial in synaptic transmission and neuroplasticity. The observed decrease in amplitude, along with altered deactivation and desensitization times, indicates that these oils may modulate glutamatergic signaling, potentially offering neuromodulation effects by reducing excitotoxicity, a major contributor to neurodegenerative diseases such as Alzheimer's and Parkinson's. In contrast, *C. pepo* exhibited minimal impact on AMPA receptor kinetics, implying a lesser role in modulating synaptic activity than the other two oils.

AMPA receptor dysregulation is implicated directly in neurodegenerative disease, with too much glutamatergic transmission leading to excitotoxicity, oxidative damage, and neuronal damage (Babaei et al., 2021). Under normal circumstances, AMPA receptors facilitate synaptic transmission, but when overactivated—most notably calcium-permeable AMPA receptors (CP-AMPARs) lacking the GluA2 subunit—neurons become highly vulnerable to excitotoxicity due to too much calcium influx (Plant et al., 2006). This influx triggers a cascade of intracellular events, including mitochondrial dysfunction, excessive reactive oxygen species (ROS) production, and activation of apoptotic pathways, all contributing to neurodegeneration (Greger et al., 2017).

In Alzheimer's disease (AD), synaptic dysfunction and cognitive decline are strongly linked to increased expression of CP-AMPARs, which disrupt calcium homeostasis in hippocampal neurons (Paoletti et al., 2013). Amyloid-beta (Aβ) oligomers have been shown to alter AMPA receptor trafficking, increasing their surface expression and exacerbating neuronal vulnerability to excitotoxicity (Babaei et al., 2021). Similarly, in Parkinson's disease (PD), glutamatergic overactivity within the subthalamic nucleus (STN) results in hyperactivation of AMPA receptors, leading to excessive calcium entry and excitotoxic damage in the substantia nigra pars compacta (Kobylecki et al., 2010). Such processes accelerate neuronal death and induce motor impairment in PD. Consistent with the outlined effects of these oils, such diminishment of the amplitude of AMPA receptors, as well as the prolongation of desensitization times, might be very helpful in softening the overactivity of CP-AMPAR, hence preventing excitotoxicity-induced cell death and synaptic decline.

Interestingly, the combined application of these oils demonstrated a synergistic effect, particularly in the case of *N. sativa* and *S. alba*. When applied together, the reduction in AMPA receptor amplitude was even more significant than when the oils were applied individually, highlighting the potential for a combined therapeutic strategy. Combining *N. sativa* with *C. pepo* or *S. alba* further reduced desensitization and enhanced deactivation times, amplifying the inhibitory effects on receptor activity. This synergy suggests that these oils interact to modulate receptor kinetics more effectively than when used alone. The most pronounced synergistic effect was observed with the combination of *N. sativa* and *S. alba*, resulting in the greatest reductions in receptor amplitude, desensitization, and deactivation times. This interaction between the bioactive compounds present in the oils could potentiate their ability to reduce glutamatergic excitotoxicity, offering enhanced neuromodulatory benefits. This synergistic modulation of AMPA receptor function aligns with these oils' known antioxidant and anti-inflammatory properties, which may further contribute to mitigating neural damage caused by oxidative stress. By combining their effects on both oxidative pathways and synaptic transmission, these oils may offer a multifaceted therapeutic approach to neurodegenerative diseases. The synergistic reduction of excitotoxic signaling, combined with their neuroprotective antioxidant actions, highlights the therapeutic potential of these natural compounds, particularly in conditions such as Alzheimer's and Parkinson's diseases, where excitotoxicity and oxidative stress are key pathological drivers.

These findings provide a foundation for further investigation into the therapeutic applications of *N. sativa*, *C. pepo*, and *S. alba* oils, especially in modulating synaptic activity and combating oxidative stress. The differential effects observed across the various biological activities suggest that these oils could be fine-tuned or combined to target specific therapeutic needs.

Further research should examine the specific AMPA receptor subtypes implicated with these oils, particularly their effect on GluA2-deficient CP-AMPARs, which are strongly implicated in excitotoxicity-mediated neurodegeneration (Plant et al., 2006; Cull-Candy et al., 2006). Electrophysiological studies of long-term potentiation and long-term depression in hippocampal and cortical networks will be critical to their role in modulating synaptic plasticity (Volianskis et al., 2015, Manahan-Volianskis et al., 2015, Wang and Reddy, 2017). Furthermore, in vivo models of Alzheimer's and Parkinson's disease should be employed to test whether these oils can retard disease progression by preserving neuronal function and cognitive performance (Wang and Reddy, 2017).

While the fixed oils of *N. sativa, C. pepo*, and *S. alba* demonstrated significant antioxidant and neuromodulatory activities in this study, their therapeutic potential is also influenced by bioavailability. The absorption, distribution, and metabolism of key bioactive compounds, such as thymoquinone, linoleic acid, and allyl isothiocyanate, are critical for their efficacy *in vivo*. Future studies should include in vitro bioavailability assessments using models like Caco-2 cell monolayers to evaluate intestinal permeability, as well as pharmacokinetic profiling in animal models to determine absorption, distribution, metabolism, and excretion (ADME). Additionally, advanced formulation strategies, such as nanoemulsions or encapsulation, could be explored to enhance the solubility and stability of these compounds, thereby improving their bioavailability. Addressing these aspects will provide a more comprehensive understanding of the therapeutic potential of these oils and facilitate their development as effective natural remedies for oxidative stress, metabolic disorders, and neurological conditions.

To build on the findings of this study, future research should focus on *in vivo* experiments to validate the antioxidant, antidiabetic, neuromodulatory, and anti-obesity effects of *N. sativa, C. pepo,* and *S. alba* oils. For antioxidant activity, *in vivo* models of oxidative stress-related diseases (e.g., neurodegenerative disorders) should be used to assess bioavailability and tissue-specific effects, alongside mechanistic studies to identify key bioactive compounds. For antidiabetic and anti-obesity potential, *in vivo* models of diabetes and obesity should be employed to evaluate the oils' effects on glucose metabolism and lipid regulation, as well as explore alternative extraction methods to enhance enzyme inhibitory activity. For neuromodulatory effects, in vivo neuroprotection studies and detailed investigations into receptor-specific mechanisms (e.g., AMPA receptor modulation) are recommended. Additionally, combination therapies with existing drugs or other natural compounds should be explored to enhance therapeutic efficacy and overcome limitations observed in single-agent treatments. These steps will provide a more comprehensive understanding of the oils' potential and their applications in treating oxidative stress, metabolic disorders,

and neurological conditions.

Actually, plants' origins and extraction methods considerably affect the bioactivity of plants' fixed oils. Cold pressing maintains heat-sensitive bio-active constituents, whereas solvent and hot-pressing extraction methods modify composition and efficacy. Geographic factors such as altitude, soil, and climate also influence fixed oils phytochemical profiles and bioactivities. Future research should focus on standardizing extraction processes, conducting *in vivo* and clinical trials to validate efficacy, and exploring novel applications in personalized medicine and drug delivery systems of the screened plants oils.

5. Conclusion

The study analyzed the antioxidant activity of *N. sativa, C. pepo,* and *S. alba* seeds fixed oils and their mixtures at different concentrations. *S. alba* oil showed the most powerful antioxidant activity, followed by *C. pepo* oil. *N. sativa* oil had the weakest antioxidant activity. The *C. pepo* and *S. alba* seeds mixture showed powerful antioxidant activity, similar to the reference compound. However, all tested oils showed weak inhibitory effects against lipase and α-amylase enzymes, suggesting weak potential for obesity treatment. No mixture showed significant inhibitory effects against amylase enzyme, suggesting weak potential for diabetic treatment. Electrophysiological studies showed that *S. alba* strongly depressed the activity of AMPA receptors by changing its kinetic properties, deactivation, and desensitization rates, which signal the neuromodulatory effect. *N. sativa* also depressed the receptor amplitudes, though to a much lesser degree, and in *C. pepo*, no significant effect on AMPA receptor activity was apparent. The wide-ranging therapeutic prospects are emphasized herein, from the antioxidant value to the possible neuromodulatory use of these oils. Further in-vitro and in-vivo testing is essential to explore their potential in developing natural pharmaceuticals.

Funding

No funds, grants, or other support was received.

CRediT authorship contribution statement

Qneibi Mohammad: Methodology, Data curation, Conceptualization. **Jaradat Nidal:** Supervision, Project administration, Data curation, Conceptualization. **Rabi Balsam:** Investigation, Formal analysis. **Faraj Haya:** Formal analysis. **Sobuh Shorooq:** Investigation, Formal analysis. **Issa Linda:** Investigation. **Hawash mohammed:** Project administration, Methodology, Investigation, Data curation, Conceptualization. **Bdir Sosana:** Resources, Methodology. **Shalabi Duha:** Formal analysis. **Idais Tala:** Investigation, Formal analysis. **Bdair Mohammad:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank An-Najah National University (www.najah.edu).

Ethics approval and consent to participate

Not applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.120868.

Data availability

Data will be made available on request.

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